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Article

Identification of Novel Benzoxa-[2,1,3]-diazole Substituted Amino Acid Hydrazides as Potential Anti-Tubercular Agents

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Abstract: Discovery and development of new therapeutic options for the treatment of *Mycobacterium tuberculosis* (*Mtb*) infection are desperately needed to tackle the continuing global burden of this disease and the efficacy and cost limitations associated with current medicines. Herein, we report the synthesis of a series of novel benzoxa-[2,1,3]-diazole substituted amino acid hydrazides in a two-step synthesis and evaluate their inhibitory activity against *Mtb* and selected bacterial strains of clinical importance utilising an end point-determined REMA assay. Alongside this, their potential for undesired cytotoxicity against mammalian cells was assessed employing standard MTT assay methodologies. It has been demonstrated using modification at three sites (the hydrazine, amino acid, and the benzodiazole) it is possible to change both the antibacterial activity and cytotoxicity of these molecules whilst not affecting their microbial selectivity, making them attractive architectures for further exploitation as novel antibacterial agents.

Keywords: benzoxa-[2,1,3]-diazole; antibacterial; *Mycobacterium tuberculosis*; amino acid; hydrazide

1. Introduction

Tuberculosis (TB) remains the predominant bacterial infectious disease globally, with approximately 10.4 million new cases and 1.7 million deaths recorded in 2016 [1]. Additionally, estimates suggest that over 20% of the population is latently infected with *Mycobacterium tuberculosis* (*Mtb*), the causative agent of TB [2]. Current treatment regimens of TB require patients to administer drug combinations for an extended period of time, at least six months, which is costly, prone to differential response rates, and exhibits significant problems with patient compliance and adherence [3]. The current treatment regimen involves combinations of rifampicin (RIF), isoniazid (INH), ethambutol (EMB), and pyrazinamide. However, resistance to these drug therapies has exacerbated the management issues of TB. In 2016 alone, almost 500,000 new cases of multidrug-resistant TB (resistant to both RIF and INH) and over 100,000 cases of RIF-resistant TB were identified [1]. Consequently, the identification of

novel therapeutic options is an absolute necessity for the management of TB in the future [4]. In this context, in recent years several new drugs and/or regimens have been reported for TB, including bedaquiline [5], delamanid [6], clofazimine [7], bedazuline-protomanid-linezolid [8]. Worryingly, resistance to several of these approaches has already been reported, primarily because of similar drug resistance mechanisms and target pathways [9,10]. Consequently, the development of new drug therapies for TB requires the discovery of new drug targets and novel structures which circumvent resistance mechanisms, whilst also enabling shorter treatment regimens.

Benzoxa-[2,1,3]-diazole, benzothia-[2,1,3]-diazole and benzotriazoles are a class of heterocycle which exhibit widespread therapeutic opportunities [11]. In addition, no studies have evaluated these compounds or their derivatives against *Mtb*. Inspired by virtual screening studies which identified simple substituted nitro benzoxa-[2,1,3]-diazoles as potential inhibitors of *Mtb* ATP phosphoribosyl transferase (HisG) and APS reductase (APSR) [12,13], we were attracted to this scaffold as a new starting point for the development of anti-TB drugs.

In this report we describe a series of novel benzoxa-[2,1,3]-diazole substituted amino acid hydrazides as selective drugs for the treatment of TB, highlighting the importance of the benzo-[2,1,3]-diazole, amino acid (AA) and the substituted aryl hydrazine (R_1), towards *Mtb* selectivity, potency, efficacy, and avoidance of toxicity against mammalian cells (Figure 1).

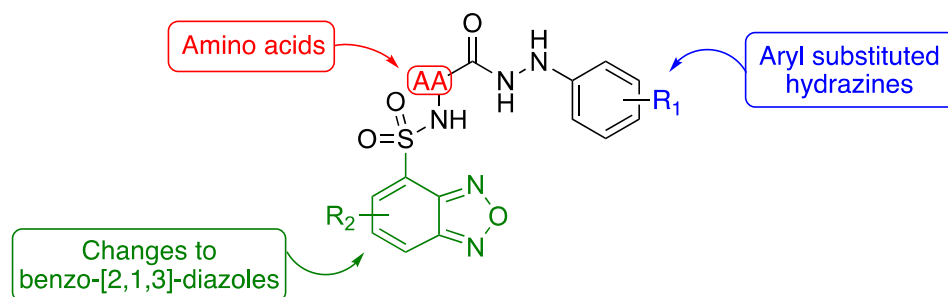


Figure 1. Inhibitors of *M. tuberculosis* based on the benzoxa-[2,1,3]-diazole framework highlighting the key modifications.

2. Results and Discussion

In the context of a study to identify novel antibacterial agents designed to overcome antimicrobial resistance, a small library of diverse bioactive compounds had previously been synthesised within our team. Using the Resazurin Microtiter Assay (REMA) [14–16], these compounds were screened for antibacterial activity at a fixed concentration (128 $\mu\text{g/mL}$) against a range of drug-susceptible bacteria including Gram-positive, Gram-negative and *mycolata* bacteria (Supporting Information, Table S1) which revealed that many possessed little utility, even at these high concentrations. However, benzo-[2,1,3]-diazole architectures 1–12 were shown to possess antibacterial activity, including activity against *mycolata* bacteria and *Mtb* (Figure 2).

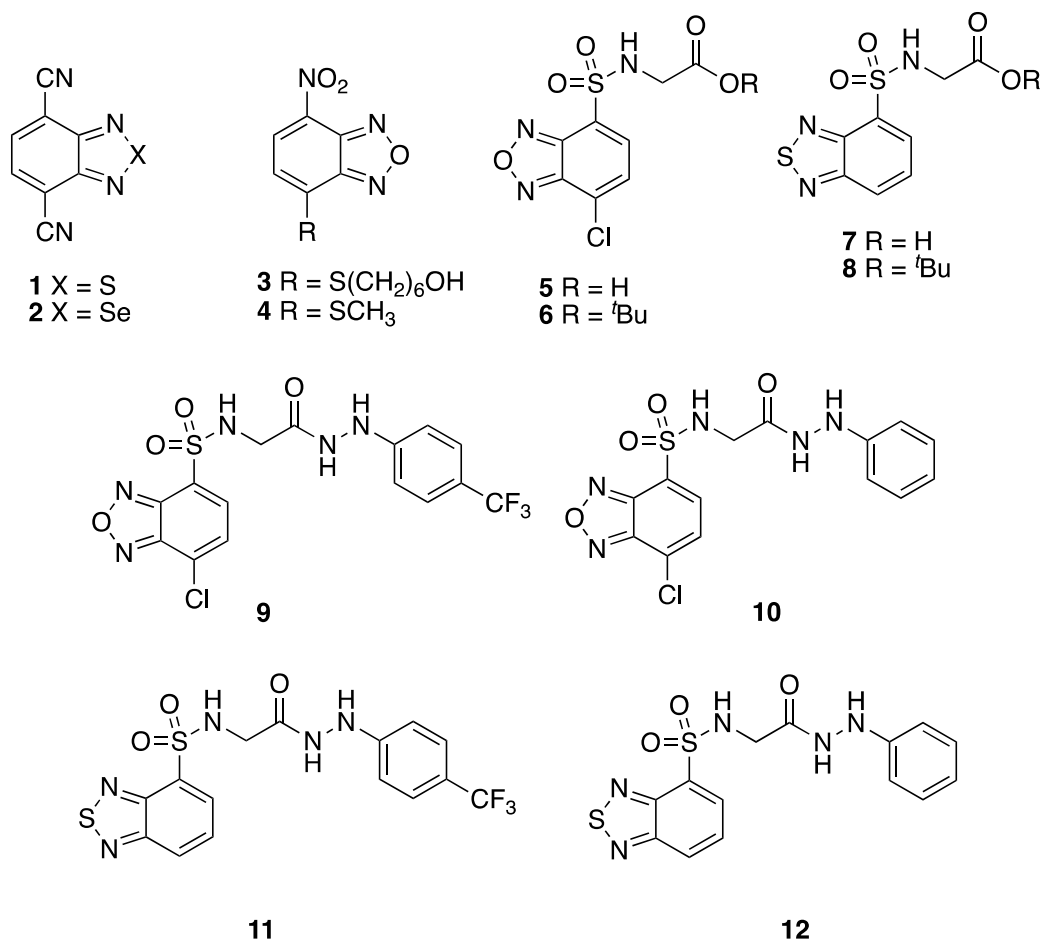


Figure 2. Benzodiazole structures from the initial 128 µg/mL screen against a range of Gram-positive, Gram-negative and *mycolata* bacteria.

To gain an improved understanding of the antibacterial potency and scope of these compounds, a dose-range REMA assay was performed (128–0.125 µg/mL, converted to µM if active) (Table 1).

Table 1. Selective antibacterial activity of benzodiazole compounds present in the compound library, expressed as mean inhibitory concentration (MIC) (µM). (–) = No activity in the REMA assay.

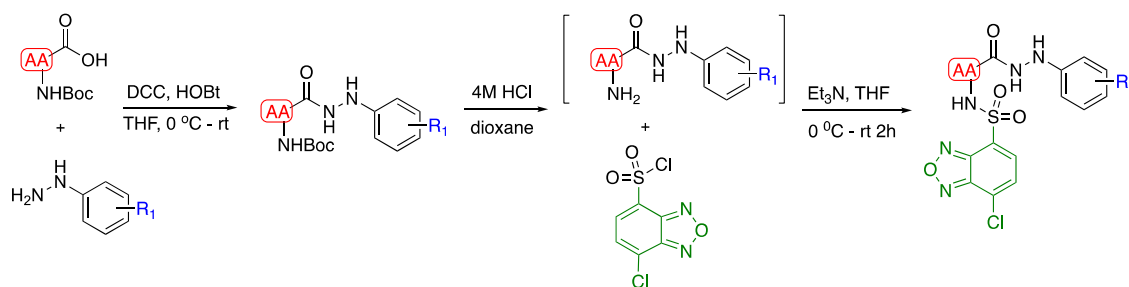
Compound	MIC (µM)						
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. equi subsp. equi</i>	<i>S. agalactiae</i>	<i>S. pyogenes</i>	<i>R. equi</i>	<i>M. tuberculosis mc²7000</i>
1	–	11.9	–	–	–	11.9	128.9
2	0.7	29.8	5.6	0.9	–	–	17.16
3	3.4	3.4	1.7	20.3	–	5.1	0.59
4	7.1	–	0.9	2.4	–	9.5	80.7
5	–	–	–	–	–	–	109.7
6	–	–	–	–	–	–	23.0
7	–	–	–	–	–	–	–
8	–	–	–	–	–	–	–
9	–	–	–	–	–	3.6	31.1
10	–	–	–	–	–	–	36.7
11	–	–	27.6	–	–	10.4	148.4
12	–	–	–	–	–	11.6	44.0
Isoniazid	–	–	–	–	–	–	0.91
Rifampin	0.12	7.78	0.03	0.97	0.49	7.78	0.03
Ethambutol	–	–	–	–	–	–	9.79

The results of the endpoint REMA assay revealed a mix of activity against the organisms tested, with simple substituted benzodiazole compounds **1–4** providing broad spectrum activity. Whilst the nitrobenzoxa-[2,1,3]-diazole **3** showed the highest levels of activity against *Mtb*, the lack of selectivity was a cause for concern. Replacing the nitro group with a sulphonamido amino acid moiety greatly improved specificity for *mycolata* bacteria, with substituted benzoxa-[2,1,3]-diazole **6** showing much higher activity than **5**, suggesting poor cell wall penetration of **5** is due to the carboxylic acid moiety. Notwithstanding this, replacement of the benzoxa-[2,1,3]-diazole with benzothia-[2,1,3]-diazoles **7** and **8** led to a complete loss of activity suggesting that the benzoxa-[2,1,3]-diazole plays a crucial role in these compounds antibacterial activity.

Further analysis of the results revealed that conversion of the ester to an aryl hydrazide **9–12** provided compounds more consistent activity across a range of structures. Consequently, substituted benzoxa-[2,1,3]-diazoles were chosen as the partner to amino acid hydrazides for further investigation, via a SAR study to further understand the importance of the amino acid (AA) and the hydrazine (R₁) on anti-mycobacterial activity.

2.1. Chemical Synthesis of Benzoxa-[2,1,3]-diazole Amino Acid Hydrazides

To undertake this investigation, a two-step synthesis was engaged starting from *N*-Boc amino acids (Scheme 1). DCC coupling with a monosubstituted hydrazine produced the intermediate protected amino acid hydrazine which, following deprotection and condensation with benzoxa-[2,1,3]-diazole sulphonyl chloride afforded the desired products in moderate to good overall yields following flash chromatography purification (Table 2). Interestingly, several of the compounds exhibited restricted rotation as demonstrated by VT-¹H-NMR spectroscopy (Supporting Information, Figure S2).

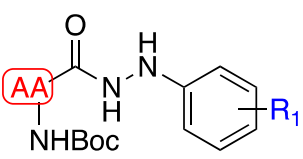
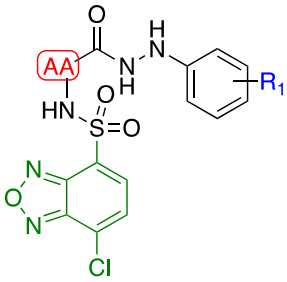


Scheme 1. Synthesis of amino acid hydrazides and the desired benzoxa-[2,1,3]-diazole amino acid hydrazides.

2.2. Structure–Activity Relationships

Following the synthetic approach, Boc amino acid hydrazides **13a–22a** and benzoxa-[2,1,3]-diazole amino acid hydrazide **9, 10, 14b–22b** were screened in the same way against the same range of drug-susceptible bacteria as described above. Importantly, in line with the preliminary screening data these compounds showed high selectivity for *mycolata* bacteria (Supporting Information, Table S3). Focusing on the *Mtb* response, initially exploring the role of the amino acid, fixing the hydrazide and increasing the bulk of the amino acid substituent **13a–17a** resulted in diminished antibacterial activity of this component (Table 2). Subsequently, fixing the amino acid to glycine, we then evaluated the role of the hydrazine component (**18a–22a**). Introduction of an unsubstituted aromatic hydrazine **18a** alongside halogenated hydrazines **19a–22a** did not provide any significant enhancement in activity although a marked increase in cytotoxicity was observed. For both series, enhanced antibacterial activity was restored on coupling to the benzoxa-[2,1,3]-diazole **9, 10, 14b–22b** albeit at the cost of increased cytotoxicity, as noted for this subunit [17].

Table 2. Results of synthesis of amino acid hydrazides and benzoxa-[2,1,3]-diazole amino acid hydrazides and the antibacterial activity and cytotoxicity.

						
13a - 22a		9, 10, 14b - 22b				
Amino Acid (AA)	Hydrazine (R ₁)	Compound	Yield	Rotamer Ratio	<i>M. tuberculosis</i> mc ² 7000 (μM)	Mammalian Cell Toxicity
Gly	4-CF ₃	13a	47%	-	12.01	0%
		9	7%	7:1	31.13	>90%
Phg	4-CF ₃	14a	95%	-	117.24	0%
		14b	63%	-	60.85	>90%
Pro	4-CF ₃	15a	85%	-	214.26	0%
		15b	85%	-	16.33	>90%
Phe	4-CF ₃	16a	83%	-	151.14	<10%
		16b	85%	-	59.27	>90%
Ala	4-CF ₃	17a	93%	-	46.06	0%
		17b	60%	-	17.25	>90%
Gly	H	18a	76%	5:1	90.46	57%
		10	60%	4:1	36.67	0%
Gly	4-F	19a	78%	4:1	112.95	59%
		19b	57%	4:1	20.00	60%
Gly	4-Cl	20a	96%	4:1	106.75	43%
		20b	43%	6:1	19.22	80%
Gly	3-Cl	21a	80%	4:1	213.50	55%
		21b	22%	5:1	19.22	45%
Gly	2-Cl	22a	95%	3:1	106.75	<20%
		22b	29%	4:1	19.22	70%

3. Discussion

Worryingly, as drug-resistant bacterial infections are on the rise and with the recent removal of antibiotic drug discovery programmes, there will be a significant demand for new chemical entities to address this condition. This study has identified that benzoxa-[2,1,3]-diazole substituted amino acid hydrazides have considerable potential as selective and potent agents against *Mtb*.

Throughout this study, the benzoxa-[2,1,3]-diazole core appears to be essential for activity. Whilst, as observed in some examples, the use of this unit is commonly associated with cytotoxicity this can be effectively modulated through the addition of the amino acid hydrazine. For example, we observed that conjugation with the amino acid hydrazides **19a** or **21a** provides a reduction in cytotoxicity (**19b**, **21b**, Table 2).

Excitingly, this modulation led to the use of a simple unsubstituted hydrazide **18a** which, although in isolation showed significant cytotoxicity, when conjugated with the benzoxadiazole **10** provides a compound with good level of activity against *Mtb* and no observable cytotoxicity.

4. Materials and Methods

4.1. Chemistry

4.1.1. Synthesis of Hydrazides—General Procedure

A solution of *N*-Boc amino acid (0.25 g, 1.43 mmol, 1 equiv.), HOBt (2 equiv.) and DCC (1.2 equiv.) was dissolved in THF (7.5 mL), cooled to 0 °C and stirred for 15 min. The solution was treated with *N*-Aryl/Alkyl hydrazine * (1.2 equiv.) before warming to room temperature and stirring for a further 1.5 h. The mixture was then poured into sat. aq. NH_4Cl (20 mL) before separating and extracting the aqueous layer with EtOAc (40 mL). The organic layer was further washed with sat. aq. NaHCO_3 (20 mL) and then brine (20 mL). The combined organic layers were dried over MgSO_4 , filtered, concentrated and dried in vacuo. Flash chromatography (DCM/EtOH/ NH_3 [600:8:1], [400:8:1], [200:8:1]) afforded the desired *N*-Boc amino acid hydrazides.

* When the hydrazine hydrochlorides were used, Et_3N (1.2 equiv.) is added to neutralise the salt

tert-Butyl 2-oxo-2-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)ethylcarbamate, **13a**

Following the general procedure outlined, *N*-Boc-L-Glycine (0.50 g, 2.9 mmol) and 4-trifluorophenyl hydrazine (0.61 g, 3.5 MMOL) was transformed following flash chromatography into the title compound which was isolated as a white solid (0.45 g, 47%); m.p. 185–187 °C; ν_{max} (ATR) 3370 (NH), 3278 (NH), 3234 (NH), 3108, 3058, 2996, 1649 (C=O), 1615, 1518 (C=O), 1330, 1245, 1156, 1107, 1052, 828, 559 cm^{-1} ; δ_{H} (700 MHz, DMSO-d_6) 9.78 (1H, s, Ar-NHNH), 8.32 (1H, s, Ar-NHNH), 7.40 (2H, d, *J* 9, Ar-*H*), 7.07 (1H, t, *J* 6, BocNH), 6.78 (2H, d, *J* 9, Ar-*H*), 3.60 (2H, d, *J* 6, NHCH_2), 1.37 (9H, s, $(\text{CH}_3)_3\text{COC(O)NH}$); δ_{C} (176 MHz, DMSO-d_6) 170.2 (CONH), 156.6 ($(\text{CH}_3)_3\text{COC(O)NH}$), 153.1 (Ar-C), 126.7 (Ar-C), 126.4 (Ar-C), 118.82 (Ar-C), 118.63 (Ar-C), 112.1 (Ar-C), 78.8 (NHCH_2), 42.7 ($(\text{CH}_3)_3\text{CO}$), 28.8 ($(\text{CH}_3)_3\text{CO}$); δ_{F} (658 MHz, DMSO-d_6) -59.30; m/z (ES^+) 356 (MNa^+), 689 ($2\text{M} + \text{Na}^+$); HRMS (ES^+) Found MH^+ , 334.13722 ($\text{C}_{14}\text{H}_{19}\text{F}_3\text{N}_3\text{O}_3$ requires 334.13730).

tert-Butyl (S)-(2-oxo-1-phenyl-2-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)ethyl)carbamate, **14a**

Following the general procedure outlined, *N*-Boc-L-phenylglycine (0.250 g, 0.99 mmol) and 4-trifluorophenyl hydrazine (0.30 g, 1.75 mmol) was transformed following flash chromatography into the title compound as a brown solid (0.386 g, 95%); R_f 0.44 (DCM/EtOH/ NH_3 [200:8:1]); m.p. 138–143 °C; ν_{max} (ATR) 3328 (N-H), 2982, 2930, 2851, 1678 (C=O), 1655 (C=O), 1524, 1322, 1158, 1113, 1067, 834, 696, 558, 488 cm^{-1} ; δ_{H} (400 MHz, CDCl_3) 8.00 (1H, bs, NH), 7.46–7.37 (7H, m, Ar-*H*), 6.68 (2H, d, *J* 8, Ar-*H*), 6.24 (1H, bs, NH), 5.69 (1H, d, *J* 7, NHCHCO), 5.35 (1H, bs, NH), 1.42 (9H, s, $\text{C}(\text{CH}_3)_3$); δ_{C} (176 MHz, CDCl_3) 157.0 (NHCHCO), 150.3 (NHCOO), 129.3 (Ar-C), 128.9 (Ar-C), 128.4 (Ar-C), 127.2 (Ar-C), 127.1 (Ar-C), 126.5 (Ar-C), 125.1 (Ar-C), 123.6 (CF_3), 112.7 (Ar-C), 80.3 ($\text{C}(\text{CH}_3)_3$), 59.4 (NHCHCO), 28.3 ($\text{C}(\text{CH}_3)_3$); δ_{F} (376 MHz, CDCl_3) -61.60 (3F, s, CF_3); m/z (ES^+) 410 (MH^+), 432 (MNa^+), 841 ($2\text{M} + \text{Na}^+$); HRMS (ES^+) Found MH^+ , 410.1689 ($\text{C}_{20}\text{H}_{23}\text{F}_3\text{N}_3\text{O}_3$ requires 410.1686).

tert-Butyl (S)-2-(2-(4-(trifluoromethyl)phenyl)hydrazine-1-carbonyl)pyrrolidine-1-carboxylate, **15a**

Following the general procedure outlined, *N*-Boc-L-proline (0.250 g, 1.16 mmol) and 4-trifluorophenyl hydrazine (0.30 g, 1.75 mmol) was transformed following flash chromatography into the title compound as a golden-brown solid (0.366 g, 85%); R_f 0.38 (DCM/EtOH/ NH_3 [200:8:1]); m.p. 116–120 °C; ν_{max} (ATR) 3265 (N-H), 2979, 2932, 2851, 1672 (C=O), 1618, 1399, 1324, 1107, 1065, 833, 591 cm^{-1} ; δ_{H} (400 MHz, CDCl_3) 8.91 (1H, bs, NH), 7.45 (2H, d, *J* 8, Ar-*H*), 6.87 (2H, d, *J* 8, Ar-*H*), 6.34 (1H, bs, NH), 4.45–4.34 (1H, m, NCHCO), 3.52–3.35 (2H, m, $\text{N}(\text{CH}_2)_3$), 2.06–1.87 (4H, m, $\text{N}(\text{CH}_2)_3$), 1.53 (9H, s, $\text{C}(\text{CH}_3)_3$); δ_{C} (176 MHz, CDCl_3) 172.2 (NCHCO), 156.1 (NCOO), 150.8 (Ar-C), 126.4 (Ar-C), 125.2 (Ar-C), 123.7 (CF_3), 112.7 (Ar-C), 81.0 ($\text{C}(\text{CH}_3)_3$), 58.4 (NCHCO), 47.2 ($\text{NCH}_2(\text{CH}_2)_2$), 28.4 ($\text{C}(\text{CH}_3)_3$), 25.5, ($\text{NCH}_2(\text{CH}_2)_2$), 24.8 ($\text{NCH}_2(\text{CH}_2)_2$); m/z (ES^+) 374 (MH^+), 396 (MNa^+), 769 ($2\text{M} + \text{Na}^+$); HRMS (ES^+) Found MNa^+ , 396.1497 ($\text{C}_{17}\text{H}_{22}\text{F}_3\text{N}_3\text{O}_3\text{Na}$ requires 396.1505).

tert-Butyl (S)-(1-oxo-3-phenyl-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)carbamate, **16a**

Following the general procedure outlined, *N*-Boc-L-phenylalanine (0.250 g, 0.94 mmol) and 4-trifluorophenyl hydrazine (0.30 g, 1.75 mmol) was transformed following flash chromatography into the title compound as a golden-brown solid (0.330 g, 83%); R_f 0.39 (DCM/EtOH/NH₃ [200:8:1]); m.p. 157–159 °C; ν_{\max} (ATR) 3324 (N-H), 3274 (N-H), 2931, 2851, 1686 (C=O), 1660 (C=O), 1520, 1328, 1156, 1103, 1067, 835, 716, 515 cm⁻¹; δ_H (400 MHz, CDCl₃) 7.93 (1H, bs, NH), 7.39 (2H, d, *J* 8, Ar-*H*), 7.36–7.32 (3H, m, Ar-*H*), 7.26–7.22 (2H, m, Ar-*H*), 6.61 (2H, d, *J* 8, Ar-*H*), 6.18 (1H, bs, NH), 5.09 (1H, bd, *J* 7, NH), 4.46 (1H, q, *J* 8, CH₂CHCO), 3.12 (2H, d, *J* 8, CH₂CHCO), 1.47 (9H, s, C(CH₃)₃); δ_C (176 MHz, CDCl₃) 171.6 (NHCHCO), 150.3 (NHCOO), 135.96 (Ar-C), 129.3 (Ar-C), 128.9 (Ar-C), 127.2 (Ar-C), 126.4 (Ar-C), 126.4 (Ar-C), 125.2 (Ar-C), 123.6 (CF₃), 112.7 (Ar-C), 80.9 (C(CH₃)₃), 54.5 (NHCHCO), 33.5 (CH₂CHCO), 28.3 (C(CH₃)₃); δ_F (376 MHz, CDCl₃) -61.56 (3F, s, CF₃); m/z (ES⁺) 424 (MH⁺), 446 (MNa⁺), 869 (2M + Na⁺); HRMS (ES⁺) Found MH⁺, 424.1847 (C₂₁H₂₅F₃N₃O₃ requires 424.1843).

tert-Butyl (S)-(1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)carbamate, **17a**

Following the general procedure outlined, *N*-Boc-L-alanine (0.250 g, 1.32 mmol) and 4-trifluorophenyl hydrazine (0.30 g, 1.75 mmol) was transformed following flash chromatography into the title compound as a dark brown solid (0.425 g, 93%); R_f 0.37 (DCM/EtOH/NH₃ [200:8:1]); m.p. 126–131 °C; ν_{\max} (ATR) 3321 (N-H), 2929, 2851, 1683 (C=O), 1664 (C=O), 1617, 1524, 1322, 1157, 1101, 1066, 830, 641 cm⁻¹; δ_H (400 MHz, CDCl₃) 8.50 (1H, bs, NH), 7.46 (2H, d, *J* 8, Ar-*H*), 6.87 (2H, d, *J* 8, Ar-*H*), 6.34 (1H, bs, NH), 5.07 (1H, bd, *J* 6, NH), 4.40 (1H, m, CH₃CH), 1.50 (9H, s, C(CH₃)₃), 1.43 (3H, d, *J* 7, CH₃CH); δ_C (176 MHz, CDCl₃) 171.7 (NHCHCO), 158.7 (NHCOO), 129.3 (Ar-C), 128.9 (Ar-C), 127.2 (Ar-C), 126.4 (CF₃), 112.8 (Ar-C), 80.9 (C(CH₃)₃), 54.6 (NHCHCO), 33.7 (CHCH₃), 28.3 (C(CH₃)₃); δ_F (376 MHz, CDCl₃) -61.55 (3F, s, CF₃); m/z (ES⁺) 348 (MH⁺), 370 (MNa⁺), 717 (2M + Na⁺); HRMS (ES⁺) Found MNa⁺, 370.1352 (C₁₅H₂₀F₃N₃O₃Na requires 370.1349).

tert-Butyl 2-oxo-2-(2-phenylhydrazinyl)ethylcarbamate, **18a**

Following the general procedure outlined, *N*-Boc-L-Glycine (0.50 g, 2.9 mmol) and phenyl hydrazine (0.30 mL, 3.1 mmol) was transformed following flash chromatography into the title compound as a white solid (0.30 g, 39%); R_f 0.1 (n-hexane/EtOAc 7:3) as a mixture of rotamers in the ratio [5:1] by NMR @ 25 °C; m.p. 120–122 °C; ν_{\max} (ATR) 3348 (NH), 3274 (NH), 2922, 2852, 1746, 1652 (C=O), 1640 (C=O), 1519, 1488, 1418, 1375, 1260, 1192, 1106, 1036, 874, 760 cm⁻¹; δ_H (700 MHz, DMSO-*d*₆) 9.59 (1H, s, NHNHPh), 9.08 (0.2H, s, NHNHPh), 7.85 (0.22H, s, NHNHPh), 7.65 (1H, s, NHNHPh), 7.17 (0.45H, t, *J* 8, Ar-*H*), 7.08 (2H, t, *J* 8, Ar-*H*), 7.02 (1H, t, *J* 5, BocNH), 6.74 (0.23H, t, *J* 8, Ar-*H*), 6.71–6.63 (3H, m, Ar-*H*), 6.61 (0.23H, m, Ar-*H*), 3.68 (0.34H, d, *J* 6, BocNHCH₂), 3.58 (2H, d, *J* 5, BocNHCH₂), 1.37 (9H, s, (CH₃)₃COC(O)NH), 1.34 (1.33H, s, (CH₃)₃COC(O)NH); δ_C (176 MHz, DMSO-*d*₆) 174.2 (CONH), 169.9 (CONH), 156.5 ((CH₃)₃COC(O)NH), 149.9 (Ar-C), 149.1 (Ar-C), 129.3 (Ar-C), 119.0 (Ar-C), 112.8 (Ar-C), 112.6 (Ar-C), 78.7 (NHCH₂), 78.5 (NHCH₂), 42.7 ((CH₃)₃CO), 31.9 ((CH₃)₃CO), 28.8 ((CH₃)₃CO), 22.8 ((CH₃)₃CO); m/z (ES⁺) 266 (MH⁺), 531 (2M + H⁺); HRMS (ES⁺) Found MH⁺, 266.24985 (C₁₃H₂₀O₃N₃ requires 266.14992).

tert-Butyl (2-(2-(4-fluorophenyl)hydrazineyl)-2-oxoethyl)carbamate, **19a**

Following the general procedure outlined, *N*-Boc-L-Glycine (0.25 g, 1.45 mmol) and 4-fluorophenyl hydrazine (0.20 g, 1.57 mmol) was transformed following flash chromatography (DCM/EtOH/NH₃ [600:8:1], [400:8:1], [200:8:1]) into the title compound as a light brown solid (0.32 g, 78%) as a mixture of rotamers [4:1] by NMR @ 25 °C; R_f 0.32 (DCM/EtOH/NH₃ [200:8:1]); m.p. 127–129 °C; ν_{\max} (ATR) 3364 (NH), 3269 (NH), 3132, 2984, 1652 (C=O), 1505, 1393, 1370, 1269, 1225, 1224, 1162, 1029, 1007 cm⁻¹; NMR data given for major rotamer δ_H (700 MHz, CDCl₃) 8.35 (1H, bs, (CH₃)₃COC=ONH), 6.89 (2H, t, *J* 17, Ar-*H*), 6.75 (2H, dd, *J* 9, 4, Ar-*H*), 5.30 (1H, bs, NHC=O), 4.07 (1H, bs, NH), 3.85 (2H, d, *J* 6, CH₂NH), 1.45 (9H, s, (CH₃)₃COC=ONH); δ_C (176 MHz, CDCl₃) 170.4 (C=O), 157.4 (C=O), 158.6 (Ar-C), 143.7

(Ar-CN^H), 115.7 (Ar-C), 114.9 (Ar-C), 81.0 ((CH₃)₃COC=ONH) 43.5 (CH₂), 28.7 ((CH₃)₃COC=ONH); δ_F (376 MHz, CDCl₃) -123.19 (Ar-F); m/z (ES⁺) 284 (MH⁺), 306 (MNa⁺), 589 (2M + Na⁺); HRMS (ES⁺) Found MH⁺, 284.1412 (C₁₃H₁₉N₃O₃F requires 284.1410).

***tert*-Butyl (2-(2-(4-chlorophenyl)hydrazineyl)-2-oxoethyl)carbamate, 20a**

Following the general procedure outlined, *N*-Boc-L-Glycine (0.25 g, 1.45 mmol) and 4-chlorophenyl hydrazine (0.22 g, 1.57 mmol) was transformed following flash chromatography into the title compound as a brown solid (0.41 g, 96%) as a mixture of rotamers [4:1] by NMR @ 25 °C; R_f 0.2 (*n*-hexane/EtOAc [1:1]); m.p. 118–120 °C; ν_{\max} (ATR) 3365, 3275 (NH), 3122, 3044, 2929, 2851, 1697, 1650 (C=O), 1525, 1491, 1441, 1391, 1369, 1311, 1268, 1241, 1158, 1091, 1053 cm⁻¹; NMR data given for major rotamer δ_H (700 MHz, CDCl₃) 8.07 (1H, bs, C=ONH), 7.17 (2H, d, *J* 9, Ar-*H*), 6.76 (2H, d, *J* 9, Ar-*H*), 5.16 (1H, bs, NH), 3.87 (2H, d, *J* 7, CH₂NH), 3.45 (1H, bs, NH), 1.47 (9H, s, (CH₃)₃COC=ONH); δ_C (176 MHz, CDCl₃) 169.0 (C=O), 156.5 (C=O), 146.2 (Ar-CCl), 129.1 (Ar-C), 126.2 (Ar-CN^H), 114.8 (Ar-C), 80.4 ((CH₃)₃COC=ONH), 43.8 (CH₂NH), 28.3 ((CH₃)₃COC=ONH); m/z (ES⁺) 300 ([³⁵Cl]MH⁺), 302 ([³⁷Cl]MH⁺), 322 ([³⁵Cl]MNa⁺), 324 ([³⁷Cl]MNa⁺), 623 ([^{35,35}Cl]2M+Na⁺), 625 ([^{35,37}Cl]2M + Na⁺), 627 ([^{37,37}Cl]2M + Na⁺); HRMS (ES⁺) Found [³⁵Cl]MH⁺, 300.1134 (C₁₃H₁₉N₃O₃³⁵Cl requires 300.1115).

***tert*-Butyl (2-(2-(3-chlorophenyl)hydrazineyl)-2-oxoethyl)carbamate, 21a**

Following the general procedure outlined, *N*-Boc-L-Glycine (0.25 g, 1.45 mmol) and 3-chlorophenyl hydrazine hydrochloride (0.28 g, 1.57 mmol) was transformed following flash chromatography into the title compound as a pale brown solid (0.34 g, 80%) as a mixture of rotamers [4:1] by NMR @ 25 °C; R_f 0.38 (DCM/EtOH/NH₃ [200:8:1]); m.p. 119.5–121.7 °C; ν_{\max} (ATR) 3343 (NH), 3269 (NH), 3077, 2980, 2932, 1659 (C=O), 1597, 1512, 1490, 1368, 1242, 1230, 1156, 1046, 1030 cm⁻¹; NMR data given for major rotamers δ_H (700 MHz, CDCl₃) 8.09 (1H, bs, NH), 7.12 (1H, t, *J* 8, Ar-*H*), 6.85 (1H, ddd, *J* 8, 2, 1, Ar-*H*), 6.81 (1H, dd, *J* 2, Ar-*H*), 6.70 (1H, ddd, *J* 8, 2, 1, Ar-*H*), 5.17 (1H, bs, NH), 3.88 (2H, d, *J* 6, CH₂NH), 1.47 (9H, s, (CH₃)₃COC=ONH); δ_C (176 MHz, CDCl₃) 170.0 (C=O), 157.5 (C=O), 148.9 (Ar-C), 135.1 (Ar-CN^H), 130.2 (Ar-C), 121.3 (Ar-C), 113.4 (Ar-C), 111.8 (Ar-C), 81.2 ((CH₃)₃COC=ONH), 28.3 ((CH₃)₃COC=ONH); m/z (ES⁺) 300 ([³⁵Cl]MH⁺), 302 ([³⁷Cl]MH⁺), 322 ([³⁵Cl]MNa⁺), 324 ([³⁷Cl]MNa⁺), 623 ([^{35,35}Cl]2M + Na⁺), 625 ([^{35,37}Cl]2M + Na⁺), 627 ([^{37,37}Cl]2M + Na⁺); HRMS (ES⁺) Found [³⁵Cl]MH⁺, 300.1119 (C₁₃H₁₉N₃O₃³⁵Cl requires 300.1115).

***tert*-Butyl (2-(2-(2-chlorophenyl)hydrazineyl)-2-oxoethyl)carbamate, 22a**

Following the general procedure outlined, *N*-Boc-L-Glycine (0.25 g, 1.45 mmol) and 2-chlorophenyl hydrazine hydrochloride (0.28 g, 1.57 mmol) was transformed following flash chromatography into the title compound as a light brown gum (0.41 g, 95%) as a mixture of rotamers [3:1] by NMR @ 25 °C; R_f 0.50 (DCM/EtOH/NH₃ [200:8:1]); ν_{\max} (ATR) 3262 (NH), 3074, 2960, 2928, 2851, 2118, 1668 (C=O), 1593, 1536, 1498, 1456, 1393, 1365, 1281, 1254, 1226, 1171, 1053, 1035 cm⁻¹; NMR data given for major rotamer δ_H (700 MHz, CDCl₃) 8.23 (1H, bs, NH), 7.31 (1H, d, *J* 8, Ar-*H*), 7.14 (1H, t, *J* 8, Ar-*H*), 6.88 (1H, dd, *J* 8, *J* 1, Ar-*H*), 6.84 (1H, t, *J* 8, Ar-*H*), 6.40 (1H, bs, NH), 3.90 (2H, d, *J* 6, CH₂NH), 3.47 (1H, bs, NH), 1.47 (9H, s, (CH₃)₃COC=ONH); δ_C (176 MHz, CDCl₃) 169.7 (C=O), 156.9 (C=O), 143.5 (Ar-CCl), 129.5 (Ar-C), 127.6 (Ar-C), 121.4 (Ar-C), 119.5 (Ar-CN^H), 113.5 (Ar-C), 80.9 ((CH₃)₃COC=ONH), 43.5 (CH₂NH), 28.2 ((CH₃)₃COC=ONH); m/z (ES⁺) 300 ([³⁵Cl]MH⁺), 302 ([³⁷Cl]MH⁺), 322 ([³⁵Cl]MNa⁺), 324 ([³⁷Cl]MNa⁺), 623 ([^{35,35}Cl]2M + Na⁺), 625 ([^{35,37}Cl]2M + Na⁺), 627 ([^{37,37}Cl]2M + Na⁺); HRMS (ES⁺) Found [³⁵Cl]MH⁺, 300.1108 (C₁₃H₁₉N₃O₃³⁵Cl requires 300.1115).

4.1.2. Synthesis of Benzoxa-[2,1,3]-Diazole Peptidomimetics—General Procedure

N-Boc amino acid hydrazides (1.20 equiv.) were dissolved in 4 M HCl solution in dioxane (3 mL) and stirred for 30 min at room temperature. The solvent was then removed in vacuo, the resulting solid was suspended in THF (3 mL) and Et₃N (3 equiv.) was added. The solution was cooled to 0 °C, before treating with 7-chlorobenzoxa-[2,1,3]-diazole-4-sulphonyl chloride (50 mg, 0.20 mmols,

1 equiv.) before warming to room temperature and stirring for 2 h. The mixture was then poured into sat. aq. NH_4Cl (20 mL). The aqueous layer was separated and extracted with EtOAc (20 mL). The combined organic layers were dried over MgSO_4 , filtered, concentrated and dried in vacuo. Flash chromatography (DCM/EtOH/ NH_3 [600:8:1], [400:8:1], [200:8:1]) afforded the desired sulphonamides.

7-Chloro-N-(2-oxo-2-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)ethyl)benzo[c][1,2,5]oxadiazole-4-sulfonamide, **9**

Following the general procedure outlined, *tert*-Butyl 2-oxo-2-(2-(4-(trifluoromethyl)phenyl)hydrazinyl)ethylcarbamate, **13a** (79 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a pale brown solid (6 mg, 7%); R_f 0.6 (DCM/MeOH 9:1) as a mixture of rotamers in the ratio [7:1] by NMR @ 25 °C; m.p. 211–213 °C; ν_{max} (ATR) 332, 3158, 1685 (C=O), 1614, 1524, 1473, 1418, 1326, 1155, 1107, 1062, 952, 834, 632 cm^{-1} ; δ_{H} (500 MHz, DMSO- d_6) 9.88 (1H, s, Ar-NHNH), 9.31 (0.13H, s, Ar-NHNH), 8.73 (1H, t, J 6, SO_2NH), 8.46 (0.14H, s, Ar-NHNH), 8.41 (0.14H, t, J 6, SO_2NH), 8.26 (1H, s, Ar-NHNH), 8.02 (1H, d, J 8, Ar-H), 7.95 (0.16H, d, J 7, Ar-H), 7.91 (0.17H, d, J 7, Ar-H), 7.85 (1H, d, J 8, Ar-H), 7.57 (0.34H, d, J 8, Ar-H), 7.43 (2H, d, J 8, Ar-H), 6.79 (0.32H, d, J 8, Ar-H), 6.68 (2H, d, J 8, Ar-H), 3.94 (0.25H, d, J 6, NHCH_2), 3.88 (2H, d, J 6, NHCH_2); δ_{C} (126 MHz, DMSO- d_6) 168.4 (C=O), 152.6 (Ar-C), 149.4 (Ar-C), 146.2 (Ar-C), 134.5 (Ar-C), 131.5 (Ar-C), 129.1 (Ar-C), 126.7 (Ar-C), 125.6 (Ar-C), 112.1 (Ar-C), 44.3 (NHCH_2); δ_{F} (376 MHz, DMSO) -59.71, -59.89; m/z (ES^-) 448 ($[\text{C}^{35}\text{Cl}]\text{M}^-$), 450 ($[\text{C}^{37}\text{Cl}]\text{M}^-$), 897 ($[\text{C}^{35}\text{Cl}, \text{C}^{35}\text{Cl}]\text{2M}^-$), 899 ($[\text{C}^{35}\text{Cl}, \text{C}^{37}\text{Cl}]\text{2M}^-$), 900 ($[\text{C}^{37}\text{Cl}, \text{C}^{37}\text{Cl}]\text{2M}^-$); HRMS (ES^-) Found $[\text{C}^{35}\text{Cl}]\text{M}^-$, 448.00931 ($\text{C}_{15}\text{H}_{10}\text{F}_3\text{N}_5\text{O}_4\text{S}$ requires 448.00996).

(S)-7-Chloro-N-(2-oxo-1-phenyl-2-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)ethyl)benzo[c][1,2,5]oxadiazole-4-sulfonamide, **14b**

Following the general procedure outlined, *tert*-butyl (S)-(2-oxo-1-phenyl-2-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)ethyl)carbamate, **14a** (97 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a pale yellow solid (66 mg, 63%); R_f 0.46 (DCM/EtOH/ NH_3 [200:8:1]); m.p. 183–187 °C; ν_{max} (ATR) 3275 (N-H), 2930, 1693 (C=O), 1618, 1521, 1325, 1161, 1105, 1067, 840, 800, 636, 619, 590, 570, 530, 504, 491 cm^{-1} ; δ_{H} (700 MHz, DMSO- d_6) 10.20 (1H, s, NH), 9.35 (1H, bs, NH), 8.36 (1H, s, NH), 7.97 (1H, d, J 7, Ar-H), 7.82 (1H, d, J 7, Ar-H), 7.32–7.27 (4H, m, Ar-H), 7.18–7.15 (3H, m, Ar-H), 6.44 (2H, d, J 8, Ar-H), 5.30 (1H, s, NHCHCO); δ_{C} (176 MHz, DMSO- d_6) 168.9 (NHCHCO), 152.3 (Ar-C), 148.9 (Ar-CNO), 145.3 (Ar-CNO), 137.1 (Ar-C), 134.9 (Ar-C), 131.1 (Ar-C), 128.9 (Ar-C), 128.5 (Ar-C), 128.3 (Ar-C), 127.9 (Ar-C), 127.7 (Ar-C), 126.4 (Ar-C), 125.6 (Ar-C), 124.5 (CF_3), 111.6 (Ar-C), 59.0 (NHCHCO); m/z (ES^+) 526 ($[\text{C}^{35}\text{Cl}]\text{MH}^+$), 528 ($[\text{C}^{37}\text{Cl}]\text{MH}^+$), 548 ($[\text{C}^{35}\text{Cl}]\text{MNa}^+$), 550 ($[\text{C}^{37}\text{Cl}]\text{MNa}^+$), 1073 ($[\text{C}^{35}, \text{C}^{35}]\text{2M} + \text{Na}^+$), 1075 ($[\text{C}^{35}, \text{C}^{37}]\text{2M} + \text{Na}^+$), 1077 ($[\text{C}^{37}, \text{C}^{37}]\text{2M} + \text{Na}^+$); HRMS (ES^+) Found $[\text{C}^{35}\text{Cl}]\text{MNa}^+$, 526.0565 ($\text{C}_{21}\text{H}_{16}\text{F}_3\text{N}_5\text{O}_4\text{S}^{35}\text{Cl}$ requires 526.0558).

(S)-1-((7-Chlorobenzo[c][1,2,5]oxadiazol-4-yl)sulfonyl)-N'-(4-(trifluoromethyl)phenyl)pyrrolidine-2-carbohydrazide, **15b**

Following the general procedure outlined, *tert*-butyl (S)-2-(2-(4-(trifluoromethyl)phenyl)hydrazine-1-carbonyl)pyrrolidine-1-carboxylate, **15a** (89 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a yellow solid (82 mg, 85%); R_f 0.46 (DCM/EtOH/ NH_3 [200:8:1]); m.p. 166–170 °C; ν_{max} (ATR) 3324 (N-H), 2929, 2851, 1682 (C=O), 1621, 1574, 1325, 1146, 1102, 1066, 946, 836, 616 cm^{-1} ; δ_{H} (700 MHz, DMSO- d_6) 10.09 (1H, s, NH), 8.39 (1H, bs, NH), 8.08 (1H, d, J 7, Ar-H), 7.86 (1H, d, J 7, Ar-H), 7.44 (2H, d, J 9, Ar-H), 6.82 (2H, d, J 9, Ar-H), 4.43 (1H, dd, J 9, 4, NCHCO), 3.61–3.57 (1H, ddd, J 10, 7, 5, $\text{NCH}_2(\text{CH}_2)_2$), 3.47–3.41 (1H, dt, J 10, 7, $\text{NCH}_2(\text{CH}_2)_2$), 1.26–0.96 (4H, m, $\text{NCH}_2(\text{CH}_2)_2$); δ_{C} (176 MHz, DMSO- d_6) 171.4 (NHCHCO), 152.8 (Ar-C), 149.5 (Ar-CNO), 146.3 (Ar-CNO), 136.8 (Ar-C), 131.2 (Ar-C), 127.7 (Ar-C), 126.5 (Ar-C), 126.3 (Ar-C), 125.5 (Ar-C), 124.7 (CF_3), 111.9 (Ar-C), 60.7 (NCHCO), 49.3 ($\text{NCH}_2(\text{CH}_2)_2$), 33.9 ($\text{NCH}_2(\text{CH}_2)_2$), 24.9 ($\text{NCH}_2(\text{CH}_2)_2$); m/z (ES^+) 490 ($[\text{C}^{35}\text{Cl}]\text{MH}^+$), 492 ($[\text{C}^{37}\text{Cl}]\text{MH}^+$); HRMS (ES^+) Found $[\text{C}^{35}\text{Cl}]\text{MH}^+$, 490.0555 ($\text{C}_{18}\text{H}_{16}\text{F}_3\text{N}_5\text{O}_4\text{S}^{35}\text{Cl}$ requires 490.0558).

(S)-7-Chloro-N-(1-oxo-3-phenyl-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide, **16b**

Following the general procedure outlined *tert*-butyl (S)-(1-oxo-3-phenyl-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)carbamate, **16a** (100 mg, 0.24 mmol) was transformed following flash chromatography into the title compound which was isolated as a brown solid (91 mg, 85%); R_f 0.41 (DCM/EtOH/NH₃ [200:8:1]); m.p. 80–84 °C; ν_{\max} (ATR) 3321 (N-H), 2932, 2854, 1677 (C=O), 1617, 1323, 1156, 1109, 1065, 834, 700, 632, 618, 577, 488 cm⁻¹; δ_H (700 MHz, DMSO-d₆) 10.13 (1H, s, NH), 9.06 (1H, d, *J* 9, NH), 8.43 (1H, bs, NH), 7.76 (1H, d, *J* 7, Ar-H), 7.71 (1H, d, *J* 7, Ar-H), 7.41 (2H, d, *J* 9, Ar-H), 7.00–6.96 (2H, m, Ar-H), 6.84–6.79 (3H, m, Ar-H), 6.70 (2H, d, *J* 9, Ar-H), 4.16–4.12 (1H, m, CH₂CHCO), 2.87 (1H, dd, *J* 14, 4, CH₂CHCO), 2.68 (1H, dd, *J* 14, 11, CH₂CHCO); δ_C (176 MHz, DMSO-d₆) 170.9 (NHCHCO), 152.6 (Ar-C), 148.9 (Ar-CNO), 144.7 (Ar-CNO), 136.9 (Ar-C), 133.9 (Ar-C), 130.7 (Ar-C), 129.5 (Ar-C), 128.2 (Ar-C), 127.6 (Ar-C), 126.5 (Ar-C), 126.2 (Ar-C), 126.2 (Ar-C), 125.5 (Ar-C), 124.6 (CF₃), 111.8 (Ar-C), 57.4 (NHCHCO), 38.0 (CH₂CHCO); m/z (ES⁺) 540 ([³⁵Cl] MH⁺), 542 ([³⁷Cl] MH⁺), 562 ([³⁵Cl] MNa⁺), 564 ([³⁷Cl] MNa⁺), 1101 ([^{35,35}Cl] 2M + Na⁺), 1103 ([^{35,37}Cl] 2M + Na⁺), 1105 ([^{37,37}Cl] 2M + Na⁺). HRMS (ES⁺) Found [³⁵Cl]MH⁺, 540.0642 (C₂₂H₁₈³⁵ClF₃N₅O₄S requires 540.0715).

(S)-7-Chloro-N-(1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)benzo[c][1,2,5]oxadiazole-4-sulfonamide, **17b**

Following the general procedure outlined, *tert*-butyl (S)-(1-oxo-1-(2-(4-(trifluoromethyl)phenyl)hydrazineyl)propan-2-yl)carbamate, **17a** (82 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a pale yellow solid (55 mg, 60%); R_f 0.34 (DCM/EtOH/NH₃ [200:8:1]); m.p. 127–131 °C; ν_{\max} (ATR) 3388 (N-H), 3315 (N-H), 3274, 2929, 2851, 1667 (C=O), 1619, 1323, 1159, 1110, 1067, 949, 836, 633, 597, 579, 511 cm⁻¹; δ_H (700 MHz, DMSO-d₆) 9.90 (1H, bs, NH), 8.80 (1H, d, *J* 8, NH), 8.24 (1H, bs, NH), 8.00 (1H, d, *J* 7, Ar-H), 7.82 (1H, d, *J* 7, Ar-H), 7.39 (2H, d, *J* 9, Ar-H), 6.61 (2H, d, *J* 9, Ar-H), 4.20–4.15 (1H, m, CH₃CHCO), 1.26 (3H, d, *J* 7, CH₃CHCO); δ_C (176 MHz, DMSO-d₆) 171.4 (NHCHCO), 152.5 (Ar-C), 149.2 (Ar-CNO), 145.6 (Ar-CNO), 134.4 (Ar-C), 131.2 (Ar-C), 128.9 (Ar-C), 128.3 (Ar-C), 126.5 (Ar-C), 125.4 (Ar-C), 124.6 (CF₃), 111.7 (Ar-C), 51.2 (NHCHCO), 19.8 (CHCH₃); m/z (ES⁺) 464 ([³⁵Cl] MH⁺), 466 ([³⁷Cl] MH⁺); HRMS (ES⁺) Found [³⁵Cl]MH⁺, 464.0400 (C₁₆H₁₄F₃N₅O₄S³⁵Cl requires 464.0402).

7-Chloro-N-(2-oxo-2-(2-phenylhydrazinyl)ethyl)benzo[c][1,2,5]oxadiazole-4-sulfonamide, **10**

Following the general procedure outlined, *tert*-butyl 2-oxo-2-(2-phenylhydrazinyl)ethylcarbamate, **18a** (64 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a yellow solid (41 mg, 55%); R_f 0.4 (DCM/MeOH 9:1) as a mixture of rotomers in the ratio [4:1] by NMR @ 25 °C; m.p. 209–211 °C; ν_{\max} (ATR) 3262 (NH), 1738, 1647 (C=O), 1606, 1530, 1494, 1410, 1346, 1190, 1157, 947, 834, 747 cm⁻¹; δ_H (500 MHz, DMSO-d₆) 9.70 (1H, s, Ar-NHNH), 9.13 (0.24H, s, Ar-NHNH), 8.68 (1H, t, *J* 6, SO₂NH), 8.35 (0.24H, t, *J* 6, SO₂NH), 8.00 (1H, d, *J* 7, Ar-H), 7.94 (0.29H, d, *J* 7, Ar-H), 7.90 (0.27H, d, *J* 7, Ar-H), 7.84 (1H, d, *J* 7, Ar-H), 7.60 (1H, s, Ar-NHNH), 7.22 (0.55H, t, *J* 7, Ar-H), 7.10 (2H, t, *J* 7, Ar-H), 6.80 (0.25H, t, *J* 7, Ar-H), 6.71–6.66 (1.6H, m, Ar-H), 6.58 (2H, d, *J* 7, Ar-H), 3.96 (2H, d, *J* 6, NHCH₂), 3.85 (2H, d, *J* 6, NHCH₂); δ_C (126 MHz, DMSO-d₆) 168.2 (C=O), 149.47 (Ar-C), 149.43 (Ar-C), 146.1 (Ar-C), 134.4 (Ar-C), 131.5 (Ar-C), 129.8 (Ar-C), 129.3 (Ar-C), 125.5 (Ar-C), 119.2 (Ar-C), 112.7 (Ar-C), 44.4 (NHCH₂); m/z (ES⁺) 382 ([³⁵Cl]MH⁺), 384 ([³⁷Cl]MH⁺); HRMS (ES⁺) Found [³⁵Cl]MH⁺, 382.03702 (C₁₄H₁₃³⁵ClN₅O₄S requires 382.03713).

7-Chloro-N-(2-(2-(4-fluorophenyl)hydrazineyl)-2-oxoethyl)benzo[c][1,2,5]oxadiazole-4-sulfonamide, **19b**

Following the general procedure outlined, *tert*-butyl (2-(2-(4-fluorophenyl)hydrazineyl)-2-oxoethyl)carbamate, **19a** (68 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a brown solid (46 mg, 57%) as a mixture of rotomers [4:1] by NMR @ 25 °C; R_f 0.48 (DCM/EtOH/NH₃ [200:8:1]); m.p. 180–185 °C; ν_{\max} (ATR) 3364, 3245 (NH), 2918, 2850, 1673

(C=O), 1507, 1453, 1414, 1367, 1346 (S=O), 1216, 1157 (S=O), 1108, 1042 cm^{-1} ; NMR data given for major rotamer δ_{H} (700 MHz, DMSO- d_6) 9.69 (1H, bs, Ar-NH), 8.63 (1H, t, J 9, CH_2NH), 7.98 (1H, d, J 7, Ar-H), 7.81 (1H, d, J 7, Ar-H), 6.91 (2H, m, Ar-H), 6.56 (2H, m, Ar-H), 3.81 (2H, d, J 6, CH_2NH); δ_{F} (376 MHz, DMSO) -126.45 (Ar-F); δ_{C} (176 MHz, DMSO- d_6) 167.9 (C=O), 155.6 (Ar-C), 149.1 (Ar-C), 145.8 (Ar-C), 145.7 (Ar-C), 134.1 (Ar-C), 131.2 (Ar-C), 129.0 (Ar-C), 125.3 (Ar-C), 115.5 (Ar-C), 113.8 (Ar-C), 44.1 (CH_2NH); m/z (ES^+) 400 ($[\text{C}^{35}\text{Cl}]\text{MH}^+$), 402 ($[\text{C}^{37}\text{Cl}]\text{MH}^+$), 422 ($[\text{C}^{35}\text{Cl}]\text{MNa}^+$), 424 ($[\text{C}^{37}\text{Cl}]\text{MNa}^+$), 799 ($[\text{C}^{35,35}\text{Cl}]2\text{M} + \text{H}^+$), 803 ($[\text{C}^{35,37}\text{Cl}]2\text{M} + \text{H}^+$), 804 ($[\text{C}^{37,37}\text{Cl}]2\text{M} + \text{H}^+$), 821 ($[\text{C}^{35,35}\text{Cl}]2\text{M} + \text{Na}^+$), 823 ($[\text{C}^{35,37}\text{Cl}]2\text{M} + \text{Na}^+$), 825 ($[\text{C}^{37,37}\text{Cl}]2\text{M} + \text{Na}^+$); HRMS (ES^+) Found $[\text{C}^{35}\text{Cl}]\text{MH}^+$, 400.0271 ($\text{C}_{14}\text{H}_{12}\text{N}_5\text{O}_4\text{FS}^{35}\text{Cl}$ requires 400.0283).

7-Chloro-*N*-(2-(2-(4-chlorophenyl)hydrazineyl)-2-oxoethyl)benzo[*c*][1,2,5]oxadiazole-4-sulfonamide, 20b

Following the general procedure outlined, *tert*-butyl (2-(2-(4-chlorophenyl)hydrazineyl)-2-oxoethyl) carbamate, **20a** (72 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a brown solid (35 mg, 43%) as a mixture of rotamers [6:1] by NMR @ 25 °C; R_f 0.37 (DCM/EtOH/ NH_3 [200:8:1]); m.p. 174–177 °C; ν_{max} (ATR) 3326, 3277 (NH), 3151, 3932, 2856, 1668 (C=O), 1523, 1491, 1325 (S=O), 1158 (S=O), 1107, 1067, 1043 cm^{-1} ; NMR data given for major rotamer δ_{H} (700 MHz, DMSO- d_6) 9.72 (1H, bs, Ar-NH), 8.62 (1H, t, J 9, NHCH_2), 7.98 (1H, d, J 7, Ar-H), 7.81 (1H, d, J 7, Ar-H), 7.72 (1H, bs, NH), 7.10 (2H, d, J 7, Ar-H), 6.55 (2H, d, J 7, Ar-H), 3.81 (2H, d, J 6, CH_2NH); δ_{C} (176 MHz, DMSO- d_6) 168.0 (C=O), 149.1 (Ar-C), 148.3 (Ar-C), 145.8 (Ar-C), 134.2 (Ar-C), 131.3 (Ar-C), 128.9 (Ar-C), 128.7 (Ar-C), 125.3 (Ar-C), 122.3 (Ar-C), 114.0 (Ar-C), 44.1 (CH_2NH); m/z (ES^+) 416 ($[\text{C}^{35,35}\text{Cl}]\text{MH}^+$), 418 ($[\text{C}^{35,37}\text{Cl}]\text{MH}^+$), 420 ($[\text{C}^{37,37}\text{Cl}]\text{MH}^+$), 438 ($[\text{C}^{35,35}\text{Cl}]\text{MNa}^+$), 440 ($[\text{C}^{35,37}\text{Cl}]\text{MNa}^+$), 442 ($[\text{C}^{37,37}\text{Cl}]\text{MNa}^+$), 853 ($[\text{C}^{35,35,35,35}\text{Cl}]2\text{M} + \text{Na}^+$), 855 ($[\text{C}^{35,35,35,37}\text{Cl}]2\text{M} + \text{Na}^+$), 857 ($[\text{C}^{35,35,37,37}\text{Cl}]2\text{M} + \text{Na}^+$), 859 ($[\text{C}^{35,37,37,37}\text{Cl}]2\text{M} + \text{Na}^+$), 861 ($[\text{C}^{37,37,37,37}\text{Cl}]2\text{M} + \text{Na}^+$); HRMS (ES^+) Found $[\text{C}^{35,35}\text{Cl}]\text{MH}^+$, 415.998 ($\text{C}_{14}\text{H}_{12}\text{N}_5\text{O}_4\text{S}^{35}\text{Cl}_2$ requires 415.9987).

7-Chloro-*N*-(2-(2-(3-chlorophenyl)hydrazineyl)-2-oxoethyl)benzo[*c*][1,2,5]oxadiazole-4-sulfonamide, 21b

Following the general procedure outlined, *tert*-butyl (2-(2-(3-chlorophenyl)hydrazineyl)-2-oxoethyl) carbamate, **21a** (72 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a pale yellow solid (29 mg, 22%) as a mixture of rotamers [5:1] by NMR @ 25 °C; R_f 0.29 (DCM/EtOH/ NH_3 [200:8:1]); m.p. 211–213 °C; ν_{max} (ATR) 3332, 3266, 3163 (NH), 2927, 2852, 1688 (C=O), 1597, 1524, 1469, 1425, 1371 (S=O), 1339, 1159 (S=O), 1073, 1043 cm^{-1} ; NMR data given for major rotamer δ_{H} (700 MHz, DMSO- d_6) 9.75 (1H, bs, Ar-CN), 8.66 (1H, t, J 6, CH_2NH), 8.10 (1H, s, NH), 7.98 (1H, d, J 7, Ar-H), 7.82 (1H, d, J 7, Ar-H), 7.08 (1H, t, J 7, Ar-H), 6.68 (1H, ddd, J 8, 2, 1, Ar-H), 6.61 (1H, t, J 4, Ar-H), 6.52 (1H, ddd, J 8, 2, 1, Ar-H), 3.82 (2H, d, J 7, CH_2NH); δ_{C} (176 MHz, DMSO- d_6) 168.13 (C=O), 150.9 (Ar-C), 149.2 (Ar-C), 145.8 (Ar-C), 134.1 (Ar-C), 131.2 (Ar-C), 130.7 (Ar-C), 128.9 (Ar-C), 125.3 (Ar-C), 118.4 (Ar-C), 114.0 (Ar-C), 111.8 (Ar-C), 111.1 (Ar-C), 44.2 (CH_2NH); m/z (ES^+) 416 ($[\text{C}^{35,35}\text{Cl}]\text{MH}^+$), 418 ($[\text{C}^{35,37}\text{Cl}]\text{MH}^+$), 420 ($[\text{C}^{37,37}\text{Cl}]\text{MH}^+$), 471 ($[\text{C}^{35,35}\text{Cl}]\text{MNa} + \text{MeOH}$), 473 ($[\text{C}^{35,37}\text{Cl}]\text{MNa} + \text{MeOH}$), 475 ($[\text{C}^{37,37}\text{Cl}]\text{MNa} + \text{MeOH}$); HRMS (ES^+) Found $[\text{C}^{35,35}\text{Cl}]\text{MH}^+$, 415.9981 ($\text{C}_{14}\text{H}_{12}\text{N}_5\text{O}_4\text{S}^{35}\text{Cl}_2$ requires 415.9987).

7-Chloro-*N*-(2-(2-(2-chlorophenyl)hydrazineyl)-2-oxoethyl)benzo[*c*][1,2,5]oxadiazole-4-sulfonamide, 22b

Following the general procedure outlined, *tert*-butyl (2-(2-(2-chlorophenyl)hydrazineyl)-2-oxoethyl) carbamate, **22a** (72 mg, 0.24 mmol) was transformed following flash chromatography into the title compound as a yellow solid (24 mg, 29%) as a mixture of rotamers [4:1] by NMR @ 25 °C; R_f 0.16 (DCM/EtOH/ NH_3 [600:8:1]); m.p. 173–175 °C; ν_{max} (ATR) 3324 (NH), 2928, 2851, 1669 (C=O), 1626, 1574, 1526, 1343, 1311, 1243, 1159 (S=O), 1088, 1042 cm^{-1} ; NMR data given for major rotamer δ_{H} (700 MHz, DMSO- d_6) 9.85 (1H, bs, Ar-NH), 8.69 (1H, t, J 6, CH_2NH), 8.00 (1H, d, J 7, Ar-H), 7.84 (1H, d, J 7, Ar-H), 7.27 (1H, s, C=ONH), 7.21 (1H, dd, J 8, 2, Ar-H), 7.10 (1H, dt, J 8, 7, Ar-H), 6.71 (1H, m, Ar-H),

6.58 (1H, dd, *J* 8, 1, Ar-*H*), 3.85 (2H, d, *J* 6, CH₂NH); δ_C (176 MHz, DMSO-*d*₆) 168.1 (C=O), 149.2 (Ar-C), 145.8 (Ar-C), 144.5 (Ar-C), 134.2 (Ar-C), 131.2 (Ar-C), 129.5 (Ar-C), 128.9 (Ar-C), 125.3 (Ar-C), 120.6 (Ar-C), 117.5 (Ar-C), 113.2 (Ar-C), 44.1 (CH₂NH); *m/z* (ES⁺) 416 ([^{35,35}Cl]MH⁺), 418 ([^{35,37}Cl]MH⁺), 420 ([^{37,37}Cl]MH⁺), 471 ([^{35,35}Cl]MNa + MeOH), 473 ([^{35,37}Cl]MNa + MeOH), 475 ([^{37,37}Cl]MNa + MeOH); HRMS (ES⁺) Found [^{35,35}Cl]MH⁺, 415.9972 (C₁₄H₁₂N₅O₄S³⁵Cl₂ requires 415.9987).

4.2. Biological Assessment

Bacterial strains and growth media used in this study (Supporting Information, Table S4).

4.2.1. Bacterial Growth Inhibition Assays

The minimum inhibitory concentration of the compounds against all strains using stand REMA assay protocols [14]. Briefly, 100 μ L of relevant growth media was added to all wells of a sterile 96-well plate (Corning Incorporated, Corning, NY, USA). The wells in rows A to H in columns 1 received 94.88 μ L of growth medium (7H9 media was supplemented with 0.2% casamino acids, 24 μ g/mL pantothenate and 10% OADC, Beckton Dickinson, Sparks, MD, USA). Compounds were added to rows A1-H1 (quadruplet per compound) followed by 1:2 serial dilutions across the plate to column 11 were 100 μ L of excess medium was discarded from the wells in column 11. The bacterial cultures at 0.5 McFarland standard diluted 1:25 was added to the wells in rows A to H in columns 1 to 11 (100 μ L), where the wells in column 12 served as drug-free controls (positive and negative). The plates were sealed with parafilmTM and incubated at 37 °C, unless 30 °C was stated as the optimum for the organism. Freshly prepared filter sterilised resazurin (0.2% *w/v*, Sigma Aldrich, Dorset, UK) was filter sterilised and 10 μ L added to all wells and re-incubated at 37 °C or 30 °C for 24 h or until the positive and negative controls showed a clear result.

4.2.2. Mammalian Cytotoxicity Determination Using the MTT Assay

In vitro chemosensitivity of Human NCI-H460 lung carcinoma cells to the agents were determined using the MTT assay, described elsewhere [18]. Cells were exposed to the amino acid hydrazides or benzoxa-[2,1,3]-diazole amino acid hydrazides (10 μ M), or solvent (dimethyl sulphoxide; DMSO) in quadruplicate. Solvent concentrations did not exceed 0.1% and were not cytotoxic. Chemosensitivity and cell survival was assessed following 96 h compound exposure, with cytotoxicity relative to vehicle control subsequently determined.

5. Conclusions

As shown by the present study, this interplay between cytotoxicity and antibacterial activity can be readily manipulated through the substitution patterns on each component, aromatic hydrazides, the size of the amino acid side chain and the benzoxa-[2,1,3]-diazole (Figure 3). The ease of manipulation makes this an attractive template and a full examination of all these parameters is the subject of ongoing efforts which will be reported in due course.

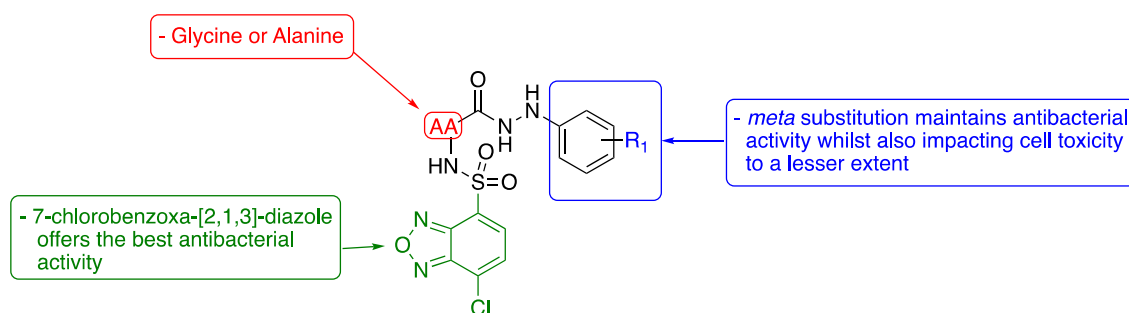


Figure 3. SAR profile of benzoxa-[2,1,3]-diazole substituted amino acid hydrazides.

Supplementary Materials: The following are available online, Table S1: Initial screen of 99 compounds against a range of Gram-positive, Gram-negative and mycolata bacterial in a 96-well plate REMA assay at 128 µg/mL. Blue cells indicate no bacterial growth. Empty cells indicate bacterial growth, Figure S1: Several of the amino acid hydrazides presented as a mixture of rotamers by NMR, Table S2: Antibacterial activity against Gram-positive, -negative and mycolata bacteria and Mammalian Cell Toxicity of amino acid hydrazides and benzoxa-[2,1,3]-diazole amino acid hydrazides, expressed as MIC (µM) or percentage relative to control (100%). - = No activity from the REMA assay at 128 µg/mL, Table S3: Bacterial strains and growth media used in the REMA assays, ¹H NMR, ¹³C NMR for all compounds.

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Sample Availability: Samples of all compounds are available from the authors.



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